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EFFET DES VERS DE TERRE EXOTIQUES SUR LES COMMUNAUTÉS DES PLANTES DE SOUS-BOIS DE FORÊT DU SUD DU QUÉBEC

par

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Le présent mémoire traite de deux approches permettant d'aborder les effets de l'invasion de vers de terre exotiques dans les forêts du sud du Québec : il s'agit d'un échantillonnage des vers de terre et des espèces herbacées de sous-bois de forêt, et d'une étude expérimentale faite en mésocosmes.

En premier lieu nous avons réalisé un échantillonnage des vers de terre et des espèces herbacées de sous-bois de forêt du parc écoforestier de Johnville. On est parti de l'hypothèse de travail selon laquelle la structure de communautés des espèces herbacées du sous-bois de forêt est déterminée par les abondances des vers de terre et en essayant de répondre à la question de savoir s'il existe une corrélation entre la structure et la composition des communautés des espèces de plantes de sous-bois et l'abondance des vers de terre du parc écoforestier de Johnville, indépendamment des variables abiotiques et historiques. Ici, nous donnerons les détails sur des résultats issus de l'analyse des données d'un échantillonnage de vers de terre et d'espèces herbacées du sous-bois de forêt réalisé sur 87 placettes dans trois zones du parc, tout au long de 18 transects perpendiculaires aux chemins se trouvant dans notre zone d'étude. On a trouvé que les vers de terre sont présents en grande quantité dans les zones Récréation et Plantation tandis qu'ils sont quasiment absents dans la zone Conservation et que les espèces herbacées du boisé de Johnville sont structurées en trois communautés réparties respectivement sur les 3 secteurs de ce territoire. Les résultats tirés des analyses statistiques montrent comme plusieurs autres études qu'il y a une association entre l'abondance des vers de terre et la structure de la végétation du sous-bois au parc écoforestier de Johnville. Contrairement à nos attentes, nous n'avons pas détecté un lien entre les perturbations anthropiques actuelles (distance des routes et sentiers) et l'abondance de vers de terre. Après avoir analysé les données de l'échantillonnage, il se trouve que l'abondance des vers de terre est différente d'une zone à l'autre. Comme la répartition des abondances des vers de terre, les espèces herbacées du parc sont structurées en respectant la zonalité de ce site, ce qui nous amène à croire que c'est l'historique de chaque zone qui est déterminant et non les abondances de vers de terre en place.

En deuxième lieu, j'ai réalisé une étude expérimentale faite en mésocosmes dans des conditions contrôlées. Les forêts du Canada et du nord des États-Unis connaissent des changements des communautés des espèces herbacées du sous-bois reliée à une invasion des vers de terre exotiques introduits à l'époque coloniale dans ces forêts qui se sont régénérées, en absence de vers de terre, au lendemain de la glaciation du Wisconsin il y a plus de 10000 ans. Dans cette étude nous essayons de comprendre si ces vers invasifs affectent les plantes herbacées de ces forêts et si oui, par quels mécanismes?

Pour comprendre ces mécanismes, nous avons privilégié l'approche par analyse des traits fonctionnels en choisissant des espèces végétales qui possèdent des traits fonctionnels qui peuvent accentuer ou diminuer les effets négatifs hypothétiques créés par des changements environnementaux provoqués par les vers de terre. Ainsi nous avons mis en place un dispositif expérimental fait de 96 mésocosmes dans lesquels nous avons transplanté 12 espèces herbacées représentatives d'une communauté de sous-bois sélectionnées selon leurs abondances pour s'assurer d'avoir suffisamment d'individus, en utilisant deux traits: (i) la position d'enracinement des plantes (2 niveaux, soit dans la matière organique, soit dans le sol minéral) et (ii) le taux de mycorhization des racines (2 niveaux, fortement mycorhizées ou faiblement mycorhizées).

Après deux saisons de croissance, seule l'espèce *Phegopteris connectilis* a répondu de façon significative à l'effet des vers de terre et sa biomasse a augmenté en présence de ceux-ci. Si l'effet des vers de terre sur les sols dans nos mésocosmes est semblable aux effets sur les sols agricoles, il est possible que l'effet positif sur cette espèce soit dû à l'augmentation de fertilité causée par l'augmentation de décomposition de la matière organique du sol. Si cela est le cas, nous ne pouvons cependant pas expliquer pourquoi les autres espèces n'ont pas répondu de la même façon.

Mots clés : Invasion de vers de terre, espèces herbacées, plantes de sous-bois, forêt.

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INTRODUCTION GÉNÉRALE

Au Canada et dans la partie septentrionale des États-Unis, il est généralement admis que la plupart des vers de terre indigènes n'ont pas survécu aux glaciations du Wisconsin. La glaciation du Wisconsin a décimé la quasi-totalité des vers de terre indigènes de cette partie du continent. La très grande majorité des espèces de vers de terre qui vivent maintenant dans le sol au Canada et les régions nordiques des États-Unis résultent des récentes introductions faites par les colons européens (Reynolds 1994). Ainsi, la majorité des espèces de vers de terre dans les parties nordiques de l'Amérique de Nord font partie de ces espèces exotiques envahissantes. La dispersion des vers de terre par les humains est la plus importante puisque la dispersion naturelle des vers de terre est très lente, avec une vitesse de propagation allant de 5 à 10 mètres par an selon la région (Addison 2009, Cameron et Bayne 2009). Les sols contenant les vers de terre, même aujourd'hui, sont surtout associés aux activités humaines (agriculture, horticulture, etc). Leur abondance relative et composition spécifique sont fortement liées au type de sol, climat, végétation, topographie, historique de l'utilisation des sols et surtout à son historique d'envahissement par des espèces exotiques (Hendrix et Bohlen, 2002).

Même si les vers de terre se trouvent de plus en plus en milieu forestier, il y a encore plusieurs forêts sans vers de terre (Hedrix et Bohelen, 2002). C'est le cas notamment des endroits qui n'ont pas été soumis à une influence humaine après la glaciation. L'intérieur des forêts semble relativement peu affecté par l'invasion des vers de terre: la probabilité d'apparition de vers de terre et l'étendue de la propagation augmentent en fonction de la proximité des routes et des aménagements (Cameron et Bayne 2009, Sackett et al, 2011; Shartelletal, 2013). On les trouve généralement sous une forte densité dans des terres agricoles, à proximité des routes et autour des lacs utilisés pour la pêche, ce qui laisse penser que leur introduction a été facilitée par les activités humaines telles que le transport par le biais des pneus ou tout simplement apportés pour être utilisés comme des appâts de pêche (Hendrix et Bohelen, 2002).

Les vers de terre sont souvent appelés des « ingénieurs écosystémiques » puisqu'ils modifient beaucoup les attributs physiques et biolniques du sol. Vu que différentes espèces végétales sont adaptées différemment à ces attributs du sol, il est possible que l'envahissement des sols forestiers par les vers de terre exotiques puisse modifier également la structure des communautés végétales. En particulier, les vers de terre modifient directement le taux de décomposition de la litière, la structure physique du sol et les communautés microbiennes du sol. L'ampleur de la perte de l'horizon organique du sol de forêt et les conséquences pour les communautés végétales de la forêt indigène dépendent de l'espèce de vers de terre (Hale et al., 2005). Cela étant, il est raisonnable de croire que l'introduction des vers dans les écosystèmes qui se sont développés sans ces espèces au lendemain de la dernière glaciation auront des répercussions importantes. L'activité des vers de terre n'est pas toujours bénéfique aux plantes. Après l'envahissement, les vers de terre modifient la structure de horizons du sol, la disponibilité des éléments nutritifs, et le biote du sol. Parmi les conséquences attribuables aux vers de terre exotiques envahissants, on trouve notamment la modification de la structure du sol forestier (McLean et Parkinson 1997a) et la réduction ou l'éradication de la couche de litière (Edwards et Bohlen, 1996). Ceci peut affecter les espèces de différentes façons; les espèces herbacées du sous-bois varient en fonction de leur placement du système racinaire dans le sol et les espèces dont les racines sont limitées à la couche organique peuvent être affectées de façon négative alors que celles dont les racines sont limitées au sol minéral peuvent être affectées de façon positive. Ensuite, d'après Wardle (2002), quand les vers de terre transforment la structure d'un sol dominé par les champignons en un sol dominé par les bactéries, les relations cruciales entre les racines et la plante sont affectées. La perte de mycorhizes peut entraîner des effets négatifs sur la fonction racinaire des plantes (Lawrence et al., 2003), la croissance des plantes (Gundale, 2002) et des assemblages de communautés végétales (Holdsworth et al., 2007). Donc, les espèces peuvent être affectées différemment selon leurs taux de mycorhization.

En connaissance de ce qui précède, nous avons voulu dans le premier chapitre de ce mémoire vérifier l'hypothèse selon laquelle l'abondance des vers de terre influence la structure des

communautés de plantes de sous-bois de forêts indépendamment des autres facteurs abiotiques et biotiques, incluant l'historique du site.

Pour ce faire, nous avons posé deux questions :

1) Existe-t-il une corrélation entre la structure et la composition des communautés des espèces de plantes de sous-bois et l'abondance des vers de terre?
2) Si oui, cette corrélation existe-elle indépendamment des variables abiotiques et historiques? Autrement dit, est-ce que cette corrélation disparait en contrôlant pour des variables abiotique et historiques?

Quand l'abondance des espèces introduites devient importante, et quand l'impact de ces espèces introduites sur ces écosystèmes devient majeur, on parle d'espèces exotiques invasives. Cependant, l'histoire de l'utilisation des terres et d'autres facteurs spécifiques du site peuvent confondre les études comparant les sites sans vers avec les sites envahis par les vers (Callaham et Blair 1999 ; Jordan et al. 1999 ; Cortez et al. 2000 ; Bohlen et al. 2004a; Hale et al, 2005).
Les vers de terre affectent directement la structure et la fonction de l'écosystème en ingérant, modifiant et mélangeant la matière organique et le sol minéral (Lavelle et al. 1998), modifiant ainsi la structure, la chimie et la biologie du sol (Devliegher et Verstraete 1997; Hale et al, 2005).
Les espèces végétales du sous-bois varient dans le placement de leurs systèmes racinaires. Certaines espèces placent leurs racines uniquement dans la couche superficielle de litière, certaines uniquement dans le sol minéral, et certaines dans les deux. Donc, il est raisonnable de croire que l'abondance des espèces végétales du sous-bois ayant ces différences dans le placement de leurs systèmes racinaires seront affectées différemment par l'invasion des vers de terre. Ensuite, nous savons que ces vers de terre modifient la structure des horizons du sol, la disponibilité des éléments nutritifs, et la faune du sol.
Les vers de terre font partie de la communauté détritivore, consommant la litière de feuilles et augmentant les taux de décomposition. L'ampleur des impacts des envahissements de vers de terre sur les sols forestiers dépend de l'assemblage des espèces de vers de terre qui envahissent le sol ainsi que de l'historique de leur utilisation (Frelich et al, 2006).
Finalement, la perte de la couche superficielle des sols forestiers cause des changements dans la communauté mycorhizienne. Les changements dans la structure et la chimie du sol et le pâturage

280 par les vers de terre conduisent aux changements dans l'abondance et de la structure des
281 communautés fongiques du sol (Johnson et al 1992; McLean and Parkinson, 1998b, 2000).
282 La perte de mycorhizes peut avoir des effets négatifs sur la fonction racinaire des plantes
283 (Lawrence et al., 2002), la croissance des plantes (Gundale, 2002) et les assemblages de
284 communautés végétales (Holdsworth et al., 2007). De plus, une augmentation de la diversité des
285 vers de terre peut entraîner une diminution de la diversité des espèces végétales en raison du fait
286 que différentes espèces de vers de terre occupent plusieurs niches dans le sol. Hale et ses
287 collaborateurs, en 2005, ont constaté une diversité végétale plus faible dans les régions où l'on
288 trouve à la fois des espèces de vers épigéiques (vivant dans la couche organique supérieure du
289 sol) et endogéniques (dans des couches organiques et minérales du sol) que dans les régions où
290 l'on ne trouve que des vers endogéniques (Hopfensperger et al., 2011). Le déclin des taux des
291 champignons en abondance ou en colonisation ou des changements dans la composition de la
292 communauté fongique mycorhizienne pourrait conduire à des changements dans les
293 communautés végétales des forêts feuillus et boréales, incluant les communautés du sous-bois
294 (Frelich et al, 2006), qui sont la cible de cette étude. Bien qu'il y ait peu d'espèces végétales
295 mycorhiziennes, comme *Arisaema triphyllum* et *Circaea lutetiana,* qui ont une relation positive
296 avec la biomasse lombricienne, un beaucoup plus grand nombre d'espèces végétales est
297 négativement corrélé avec la biomasse lombricienne (Hale 2004). Donc, nous savons que les
298 structures physique, chimique et microbiolnique des sols forestiers sont changées suite à
299 l'invasion des vers de terre. Plusieurs auteurs ont affirmé que les vers de terre influencent
300 fortement la composition de la communauté végétale herbacée (Drouin *et al*, 2014, Frelich *et al*,
301 2006). Typiquement, ces études démontrent une association entre l'occurrence ou abondances
302 des vers de terre et un changement dans la structure de la communauté végétale.

303

304 Cependant, une association entre la distribution des vers de terre et la structure de la végétation
305 de sous-bois n'est pas nécessairement une relation causale puisque la corrélation entre les deux
306 peut être une réponse commune aux perturbations humaines, même pour des perturbations qui
307 datent de plusieurs décennies. Par exemple Beauséjour *et al* (2015) ont conclu que certaines
308 espèces étaient distribuées en tenant compte non seulement de la distribution spatiale des
309 perturbations humaines, mais aussi du délai après lequel la perturbation s'est répandue. Notre

premier chapitre nous a permis de conclure que la distribution des vers de terre et la structure des communautés d'espèces de sous-bois de forêt du parc écoforestier de Johnville suivent l'historique de l'utilisation de ce site. Pourtant, il existe de bonnes raisons pour supposer que l'invasion des vers de terres des sols forestiers provoque un changement de la structure et la composition des communautés végétales du sous-bois.

Il y a deux faiblesses importantes dans toutes ces études. D'abord, ces études se basent sur des corrélations entre la présence/absence (ou abondance) des vers de terre et des changements de composition des espèces végétales. Par contre, nos forêts sont bousculées simultanément par plusieurs changements : l'aménagement forestier, les changements dans les populations d'herbivores (surtout les cerfs de Virginie), les dépositions azotées et les changements climatiques. Il est très difficile de séparer les effets des invasions des sols forestiers par les vers de terre de ces autres changements en utilisant les études observationnelles. Ensuite, même si nous pouvions attribuer un effet causal aux vers de terre, il est probable que cet effet sera différent sur différentes espèces végétales. Certaines espèces végétales peuvent être favorisées, et d'autres affectées négativement. Comment prévoir ces effets? Pour démontrer une relation causale entre l'introduction des vers de terre et des changements dans la composition de la communauté des plantes de sous-bois, il faudrait faire une expérience sur le terrain en introduisant des vers de terre dans un sol qui n'a jamais eu des vers de terres, avec un contrôle statistique approprié, et ensuite suivre les changements dans la végétation. Une telle expérience n'a pas été faite et ne se fera probablement jamais à cause des contraintes d'éthique environnementale.

Dans notre chapitre 2, nous proposons un compromis entre une telle expérience idéale et des études purement observationnelles : une expérience dont on manipule la présence/absence des vers de terres mais dans des conditions plus contrôlées qui ne mettent pas en danger les sols forestiers. En plus, nous avons privilégié l'approche par analyse des traits fonctionnels en choisissant des espèces végétales qui possèdent des traits fonctionnels qui peuvent accentuer ou diminuer les effets négatifs hypothétiques créés par des changements environnementaux provoqués par les vers de terre. L'arrivée des vers de terre dans un sol forestier cause la réduction

de la couche de feuilles mortes (litière) sur la surface du sol, la couche de la matière organique décomposée des sols forestiers. Puisque plusieurs espèces de sous-bois gardent leur système racinaire dans cette couche, on peut supposer que ces espèces seront négativement affectées par les vers de terre. Les espèces dont les racines se trouvent dans le sol minéral ne devraient pas être affectées autant.

Ensuite, nous savons que les vers de terre détruisent des hyphes fongiques, incluant des hyphes mycorhiziennes (Pelosi et al, 2014) présumément en les mangeant. Si c'est bien le cas, les espèces de sous-bois qui sont plus fortement mycorhizées devraient être plus affectées négativement par les vers de terre. Existe-t-il des attributs morpholniques, physiolniques ou phrénolniques (« traits fonctionnels ») des espèces végétales qui peuvent prédire leurs réponses face aux vers de terre ? Pour approcher cette problématique, nous avons fait une expérience dans des mésocosmes. Nous y avons transplanté des espèces de sous-bois ayant des trais fonctionnels variés. Certaines espèces ont uniquement des racines dans la litière et d'autre dans le sol minéral. Dans chacune de ces deux catégories, la moitié des espèces sont fortement mycorhizées les autres faiblement. La moitié des mésocosmes a fait l'objet d'un ajout des vers des terres tandis que l'autre moitié des mésocosmes a fait l'objet d'une exclusion des vers de terres.

Ainsi dans ce deuxième chapitre nous avions deux hypothèses de travail: (1) les espèces ayant leurs racines uniquement dans la couche de litière seront affectées négativement par les vers de terre exotiques; (2) les espèces qui sont fortement mycorhizées seront aussi affectées négativement.

CHAPITRE 2

La structure d'une communauté végétale herbacée de sous-bois de forêt et sa relation avec l'abondance des vers de terre invasifs.

Titre original: The structure of a herbaceous understory plant community and its relationship to invasive earthworm abundance. (*sera soumis à Botany*)

Le présent chapitre présente les résultats issus de l'analyse des données d'un échantillonnage de vers de terre et d'espèces herbacées du sous-bois de forêt réalisé sur 87 placettes dans trois zones du parc écoforestier de Johnville au long de 18 transects perpendiculaires aux chemins.

On a trouvé que les vers de terre sont présents en grande quantité dans les zones Récréation et Plantation tandis qu'ils sont quasiment absents dans la zone Conservation et que les espèces herbacées du boisé de Johnville sont structurées en trois communautés réparties respectivement sur les 3 secteurs de ce territoire.

Les résultats montrent comme plusieurs autres études qu'il y a une association entre l'abondance des vers de terre et la structure de la végétation du sous-bois au parc écoforestier de Johnville. Comme la répartition des abondances des vers de terre, les espèces herbacées du parc sont structurées en respectant la zonalité de ce site. On est amené à penser que c'est l'historique de chaque zone qui est déterminant, en contraste avec l'affirmation selon laquelle la réponse de la forêt est fortement corrélée au type d'espèce de vers de terre présents.

385 **The structure of a herbaceous understory plant community and its relationship to invasive**
386 **earthworm abundance**

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Abstract

We asked if the community structure of forest understory herbaceous species is determined by earthworm abundances independently of abiotic and historical variables. To answer this question, we studied the structure and community composition of understory plant species and the earthworm abundance of Johnville Ecoforest Park (Quebec). Here we report results from a sampling of earthworms and herbaceous forest understory species on 87 plots in three zones of the park that differ in historical land use, along 18 transects perpendicular to the roads. Earthworms are present in large quantities in the Recreation and Plantation zones, while they are nearly absent in the Conservation zone. The herbaceous species are also structured into three communities distributed respectively over the three zones of this territory and there is an association between the abundance of earthworms and the structure of herbaceous understory vegetation. The statistical significance of this association disappears once we take into consideration the differences between land use zones. There is no link between current anthropogenic disturbances and the abundance of earthworms. The observed association between the abundance of earthworms and the structure of herbaceous understory vegetation is not causal but generated by the common response of each to land use history.

Keywords: Invasive earthworms, forest understory plant communities.

1. Introduction

In Canada and the northern United States, it is generally accepted that most native earthworms did not survive the Wisconsin glaciations. Rather, the vast majority of earthworm species now occurring in Canada and the northern United States are the result of recent introductions by European settlers (Reynolds 1994). In Quebec (Canada), 17 of 19 known earthworm species have been introduced by European settlers (Reynolds and Reynolds 1992; Addison 2009, Drouin et al. 2014). Among the exotic earthworm species, the most abundant are those of the genus *Lumbricus* (Reynolds 1977; Addison 2009). These exotic species are found mainly in agricultural land or other places disturbed by humans but are also found in some forest soils in this region (Hale et al. 2005, Bohlen et al. 2004a, Burtelow et al. 1998).

In historically worm-free soils in North America, the invasion of European earthworms is strongly linked to human activities, leading to their introduction into new areas where their establishment and spread will depend on their habitat (Kalisz and Dotson 1989, Suarez et al. 2006, Tiunov et al. 2006, Cameron et al. 2007). This human dispersal is important because the natural dispersion of earthworms is very slow, with a propagation rate ranging only up to 5 to 10 meters per year depending on the region (Addison 2009, Cameron and Bayne 2009). Soils containing earthworms, even today, are mainly associated with human activities (agriculture, horticulture, etc.). Their relative abundance and species composition are strongly related to soil type, climate, vegetation, topography, land use history and especially its history of invasions by exotic species (Hendrix and Bohlen 2002). Although earthworms are increasingly found in forest environments in these areas, there are still many forested areas without earthworms (Hendrix and Bohlen 2002). Forest interiors are often relatively unaffected by earthworm invasion; the probability of earthworm establishment and the extent of spread increases with proximity to roads and other human developments (Cameron and Bayne 2009, Sackett et al. 2011; Shartelletal 2013). In forests, they are generally found in high density near roads and around lakes used for fishing, suggesting that their introductions have been facilitated by human activities such as transport by vehicles or simply brought in to be used as fishing bait (Hendrix and Bohlen 2002). Drouin et al. (2016) confirmed that the distribution and abundance of exotic earthworms observed in forests reflect human

accessibility to these environments and that their dispersal is facilitated by humans because earthworms have been found in unprotected sites and crossed by trails and no worms have been found on protected sites or sites remote from trails and dwellings. The authors of this study also suggested that the consequences of the growing spread of earthworms in temperate forests could compromise the integrity of biodiversity and the recruitment of shrub species. The authors of the study found a negative correlation between earthworm abundance and the abundance of some plant species. However, it is also known that the history of site use can explain the distribution and dispersal rate of earthworms. Beauséjour et al. (2015) concluded that earthworm distributions are determined by not only the current spatial distribution of human disturbances, but also the history of such human disturbances. Some of the oldest human disturbances on their study site (Mont Saint Hilaire, Quebec) that are up to one hundred and fifty years old, are now barely evident in the vegetation structure, but their influence on earthworm distributions persists to this day. Therefore, the history of forest sites (former agricultural and non-agricultural land) must be taken into account to understand the current distribution of earthworms in these forest soils.

Earthworms are often referred to as "ecosystem engineers" because they significantly alter the physical and biolnical attributes of the soil. Since different plant species are adapted differently to these soil attributes, and since these North American plant communities have developed since the end of the Wisconsin glaciations in the absence of earthworms, it is possible that the invasion of forest soils by exotic earthworms may also alter the structure of these forested plant communities. In particular, earthworms directly modify the rate of litter decomposition, the physical structure of the soil and the microbial communities of the soil. The extent of loss of the organic horizon of forest soil and the consequences for plant communities of native forest depends on the invasive earthworm species (Hale et al. 2005). However, it is reasonable to believe that the introduction of worms into ecosystems that developed without these invasive species in the aftermath of the last glaciation more than 10,000 years ago will have significant impacts. The invasion of exotic earthworms into temperate and boreal forests in North America, long ignored, has become a growing concern in the scientific community (Hale et al. 2005; Suarez et al. 2006; Addison 2009).

The activity of earthworms is not always beneficial to plants. After invasion, earthworms modify the structure of soil horizons, nutrient availability, and soil biota. The type and magnitude of these impacts vary with lumbricid species and soil characteristics (Frelich et al. 2006). Earthworms are key members of the soil macrofauna and they can affect the composition of primary producers and ecosystem productivity by changing seedbed conditions, soil characteristics, water flow, nutrients and carbon, and plant relationships. There is indirect evidence that they strongly influence the composition of the herbaceous plant community. Changes in the tree seedling community attributed to exotic earthworms suggest that the composition of the tree layer will eventually change (Drouin et al. 2014, Frelich et al. 2006). However, an association between earthworm distribution and understory vegetation structure in the field is not necessarily a causal relationship since the correlation between the two can be a common response to human disturbances, even for disturbances that date back several decades. However, there are good reasons to assume that the invasion by earthworms of forest soils causes a change in the structure and composition of understory plant communities. Consequences attributable to invasive exotic earthworms include changes in forest soil structure (McLean and Parkinson 1997) and reduction or eradication of the bedding layer (Edwards and Bohlen 1996). This can affect species in different ways; herbaceous species in the understory vary according to their placement of the root system in the soil and species whose roots are limited to the organic layer might be negatively affected while those whose roots penetrate into the mineral soil might be positively affected. Furthermore, according to Wardle (2002), when earthworms transform the structure of a fungus-dominated soil into a bacteria-dominated soil, the relationships between the roots and the plant are affected. The loss of mycorrhizae can have negative effects on the root function of plants (Lawrence et al. 2003), plant growth (Gundale, 2002) and plant community assemblages (Holdsworth et al. 2007). Therefore, species may be affected differently depending on their mycorrhization rates.

In this respect, we wanted to test the hypothesis that the abundance of earthworms influences the structure of forest understory plant communities independently of other abiotic and biotic factors, including the history of the site. To do this, we asked two questions:

1) Is there a correlation between the structure and community composition of understory plant species and the abundance of earthworms?

2) If so, does this correlation exist independently of abiotic and historical variables? In other words, does this correlation disappear by controlling for abiotic and historical variables?

2. Materials and Methods

Presentation of the study area

The sampling campaign was conducted in the summer of 2017 at Johnville Ecoforest Park (45.345° N, 71.755° W) in the Estrie region of south-central Quebec, Canada. This park covers 224 ha, 85 of which is a peat bog (Grégoire et al. 2010), the rest being a mixed forest located about 300 km south of the boreal forest. The dominant tree species are *Acer rubrum* Linnaeus, *Betula papyrifera* Marshall, *Abies balsamea* Linnaeus, *Betula alleghaniensis* Britton. The park prohibits activities that could have a negative impact on wetlands and forests, such as drainage, road construction, motorized traffic, among others except for some recreational and educational activities intended for the general public (L. DeSerres, personal communication). This study site is located in an area that receives up to 1209 mm of precipitation per year and average temperatures range from -10.2°C in January to 19.0° in July (Environment Canada 2018).

Sampling

Sampling was carried out at three sectors of the park that we will call "zones": the Plantation zone (P) in the northeast, the Conservation zone (C) in the southeast and the Recreation zone (R) in the southwest,which shelters the Lavigne spring that has supplied water to the villages surrounding the site since 1919. The "P" zone was planted before the 1900s, mainly with forest species such as white pine, red pine and maple. The "R" zone is a secondary forest that has developed on former agricultural fields (Figure 1).

524

525

526 **Figure 1. 1945 aerial photo of Johnville Ecoforest Park.**

527 (source: Department of Energy, Mines and Resources, Canada). Flight line: A9429, Photo no. 56,
528 Latitude: 45°20'14", Scale: 1/20 000. The red outline determines the approximate location of the
529 park.

530

531 Zone "C", which is located around the lakes, one of which has historically provided drinking water
532 for the village of Lennoxville, does not appear to have ever experienced significant anthropogenic

pressure. Indeed, the road leading into this part of the park was built with local materials around 1969.

We collected our samples from 87 plots of 50 cm X 50 cm each, along 18 transects perpendicular to the roads or trails of the park. On the same path, in the same sector, the transects were parallel to each other and equidistant by 50 m. For a given transect, the plots were placed 1m, 6m, 16m, 66m, and 116m from the road or path. We used this sampling design since many studies have shown that earthworm density often decreases as one moves away from a road (Cameron and Bayne 2009; Hale et al 2008). In each plot, herbaceous species were identified and the abundance of each was estimated by counting the number of stems. The number of earthworms per plot was quantified using Lawrence and Bowers' (2002) mustard solution extraction method, in which a mustard solution (10g/l) is poured on the soil previously cleared of litter and vegetation after counting the number of herbaceous plant individuals. This solution causes earthworms to return to the surface. All individuals observed for 15 to 20 minutes after application of the mustard solution were counted. Lawrence and Bowers (2002) showed that this method is as effective as other methods while being much faster. Since we could not identify juvenile individuals to species, we worked only with the total abundance of earthworms. Environmental data were collected on the following variables: (1) the thickness of the litter layer (in cm) was measured using a ruler; (2) soil drainage was estimated from the absorption rate of the mustard solution by the soil and classified into three categories. After pouring 5 liters of mustard solution onto the quadrat, drainage was deemed rapid if it disappears within 5 min, moderate for 5-10 min and slow for 10 and more; (3) soil texture was classified into the four categories (sand, silt, clay and organic matter) assessed taking into account the most dominant constituent element. These elements have been classified according to a rapid test as described by Saucier (1994, pp. 32-35); (4) Percent canopy cover was estimated using a digital photo taken by a camera equipped with a hemispherical lens and the free LGA (Gap Light Analyzer) software from the Cary Institute of Ecosystem Studies (www.caryinstitute.org). The DBH (diameter at breast height) was measured with a tape on tree stems larger than 2cm to deduce basal area. The DBH was been estimated in a circle of 2m radius with the center of the plot.

562 **3. Statistical analyses**
563

564 All statistical analyses were performed with R software (version 3.4.3). The relationship between
565 earthworm density and environmental conditions was determined using generalized mixed linear
566 models using the lmer function (lme4 library) with transect as a random variable and using the
567 quasi-Poisson distribution in our model to manage cases of overdispersion (Crawley 2012). We
568 tested the statistical significance of the relationships in these generalized linear models between
569 earthworm abundance and environmental variables such as distance from plots to roads, zone
570 (zoning in park sectors), percent canopy, basal area, bedding thickness, soil drainage, number of
571 stems and soil texture with ANOVAs by using the chi-square distribution and comparing nested
572 models (with/without the explanatory variable).

573 The relationships between the structure of understory vegetation and environmental variables were
574 determined using redundancy analysis (Legendre and Legendre 2012) using the vegan package
575 (version 2.4.6). We constructed a matrix of the relative abundances of each herbaceous species
576 (response variable) found in each quadrat. To do this, we used a Hellinger transformation from
577 abundances per plot to relative abundances (Legendre and Gallagher 2001). A Hellinger
578 transformation is available in the vegan library of R under the decostand function (). This
579 transformation is appropriate for our species abundance data because the proportion of zeros in the
580 matrix is high (92%), and this avoids double zeros being considered in the calculation of similarity
581 between plots (Legendre and Gallagher 2001). This produces a matrix (V) that describes the
582 relative abundance of each plant species in each quadrat. Next, we constructed a matrix of
583 explanatory variables (E). This matrix describes the values of each environmental variable,
584 including the zone in which the plot is found, plus the abundance of earthworms found in each
585 quadrat. Having different units, the environmental variables in matrix E were standardized before
586 calculating the distance measurements used to perform the ordination analyses (Borcard et al.
587 2011). Some variables were ln-transformed before analysis as ln(x) or ln(x+1) on a case-by-case
588 basis to ensure that their residuals were distributed according to a normal distribution. The
589 abundance of earthworms was also included in matrix E; these abundances were transformed into
590 ln(x+1), where x is the number of individuals recorded. We related the vegetation (V) and
591 environmental E matrices using redundancy analysis (RDA) via the RDA function of the vegan

library. Null probabilities, associated with these models, were determined by permutation tests, using 1000 permutations. The analysis was done in three steps:

Step 1: We tested the null hypothesis that there is no association between earthworm abundance and vegetation structure, without reference to other environmental variables, using an RDA that relates the matrix V to only the transformed abundance of earthworms. The rejection of this null hypothesis demonstrates that there is an association between the abundance of earthworms and the structure of vegetation.

Step 2: An RDA was then performed using all environmental variables without the abundance of earthworms. We retained the significant explanatory variables by progressive selection using the vegan ordiR2step function. A significant relationship shows that there is an association between some of these environmental variables and vegetation structure.

Step 3: Then the RDA was redone using the environmental variables selected in Step 2 plus the transformed abundances of earthworms. If the structure and composition of vegetation is affected by earthworm abundance independently, or in addition to other environmental variables, then this variable will have a significant association in the model. If, on the other hand, the association between the structure and composition of vegetation and earthworms is caused only by a common response to other environmental variables, then the abundance of earthworms will lose its significance in this model.

4. Results

Earthworm community

A model linking the abundance of earthworms according to the three zones shows that the abundance of earthworms differs from one zone to another ($p<0.001$). Earthworms are present in large quantities in the Recreation and Plantation zones while they are almost absent in the Conservation zone. We observed only one earthworm in the Conservation zone while we observed 637 and 583 earthworms in the Recreation and Plantation Zones, respectively (Figure 2).

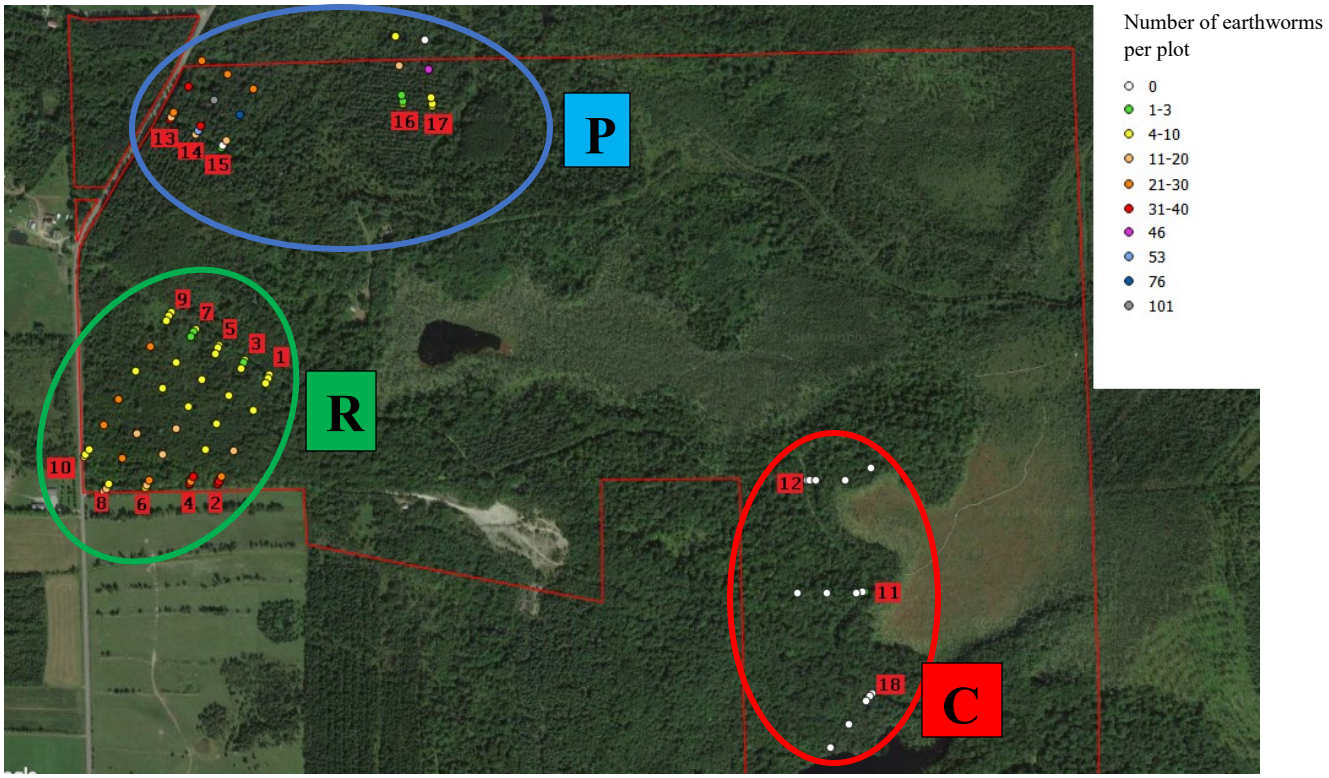

Figure 2. Sampling plan and illustration of earthworm abundances per plot.

Google aerial image/ unknown year (Municipalité régionale de comté du Haut St-François), P = Plantation, R = Recreation, C = Conservation, the numbers 1 to 18 represent the number of each transect.

Contrary to our hypotheses, earthworms, in the two zones (R and P) where they are abundant, are not distributed according to any environmental variable. Their abundance is not significantly correlated to any of the response variables (bedding thickness, soil texture, drainage, moisture, DBH, canopy opening, basal area), including distance from a source of human disturbance, whether a road or a walking trail.

Influence of earthworm abundance on the structure of forest understory plant communities

A redundancy analysis (RDA) of the structure (V) of the herbaceous community as a function of earthworm abundance shows that the two are significantly associated ($F_{1,85} = 3.1352$, $p = 0.001$). However, in the stepwise selection of variables, the only environmental variables selected that had

a significant association with the structure of the understory herbaceous communities were the zone in which the plot was found. In particular, the redundancy analysis of the structure (V) of the herbaceous community according to zone (Z) shows that the latter has a significant response ($F_{1,85}$ = 4.6439, p = 0.001). Thus, the vegetation structure of the understory is associated with both the zone and the abundance of earthworms. On the other hand, there is also a strong association between the zone and the abundance of earthworms. A third redundancy analysis (RDA) of the structure (V) of the herbaceous community as a function both of the abundance of earthworms and the zone together shows that earthworms do not have a significant response ($F_{1,84}$ = 0.9439, p = 0.5246) by controlling for the zone but the zone remains significant ($F_{1,84}$ = 2.3979, p = 0.0013) when controlling for earthworm abundance. Figure 3 summarizes the RDA and shows that the herbaceous species of the Johnville woodland are structured into 3 communities spread over the 3 sectors of this territory.

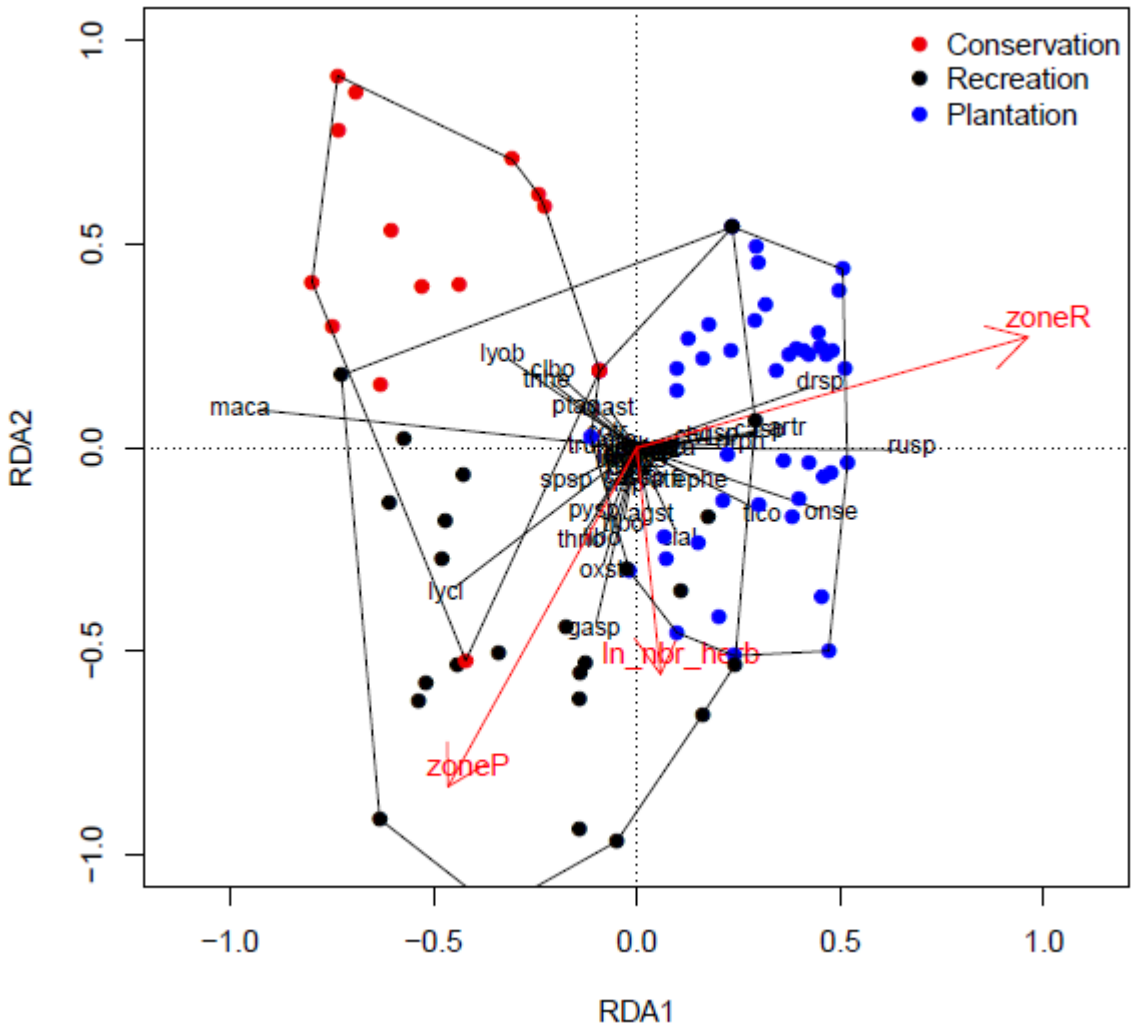

Figure 3. Ordination of understory plant community from Johnville ecoforest park.

The output of an RDA shows 3 distinct zones (Conservation zone in red, Recreation zone (R) in black and Plantation zone (P) in blue. The meaning of the plant species codes is given in the appendix.

5. Discussion

In our study, as in several other studies (for example, Frelich et al. 2006; Drouin et al, 2016), there is an association between the abundance of earthworms and the structure of understory vegetation. Contrary to our expectations, we did not detect an association between current anthropogenic

disturbances (distance from roads and trails) and the abundance of earthworms. However, there is a very clear association between the historical use of the site and the abundance of earthworms. At Johnville Park, the three sectors (zones) have experienced different human activities in the past. The Plantation zone is currently occupied by conifers originally planted on an old farm. The Recreation zone, dominated by yellow birch, is a younger secondary forest that has also developed on former agricultural land. These two zones have experienced agricultural activities in the past and anthropogenic pressure has been further encouraged by the alignment of access roads for water intake work from the site's source to supply water to surrounding villages, including the current town of Lennoxville. This activity dates back to 1919 (History of the Johnville Woodlands, 2002), i.e. nearly 100 years ago, before which there were agricultural activities. Agricultural activities are pointed out because they have a great potential to be associated with the spread of earthworms, which could partly explain the presence and high density of earthworms in these zones. The Conservation zone remained isolated until 1979 when a road was built to access it. This may partly explain the very low density of earthworms (1 worm sampled throughout the area) compared to the other two zones. It is also important to note that the only earthworm in this zone was sampled close to the road, at 1 meter. This indicates that this zone remains well preserved and that the introduction of worms into this part of the park is probably recent.

There is an additional reason that could explain this uneven distribution of earthworm densities at the study site. According to information gathered from current park managers, roads and hiking trails in the Recreation and Plantation sectors have been developed with materials collected elsewhere, potentially containing earthworms, while for roads in the Conservation Zone, the materials used have been dug on site in the zone itself. The Conservation Zone has been lnged in the past but does not have an agricultural past. Since the natural dispersion of earthworms is very slow, at most 10 meters per year (Addison 2009), the most likely explanation is that earthworms were introduced into the Recreation and Plantation zones by agricultural activities prior to 1919, the year the site was acquired by the Town of Lennoxville, and that earthworms have not yet invaded the Conservation Zone.

Although the current distribution of earthworms and understory vegetation structure appears to follow the history of the site, does this necessarily mean that earthworms do not have an effect on vegetation structure? Indeed, there is a considerable spatial variation in the abundance of earthworms within the Recreation and Plantation sites, ranging from 0 to 404 individuals per m^2 (101 worms being the largest count on a 0.25 m^2 plot). On the other hand, by including the zone and abundance of earthworms in the RDA, the abundance of earthworms lost its statistical significance, but the zone continued to be significant. This suggests that the association between earthworm abundance and vegetation structure was due to the fact that both respond simultaneously to differences in historical use of the site (zoning). Within each zone, the variation in earthworm abundance is not correlated with differences in understory vegetation structure. Like the distribution of earthworm abundances, the herbaceous species in this park are structured according to the land use history of the park, contrary to our working hypothesis that this structure would be determined by the abundances of earthworms. Here too, we are led to believe that it is the past of each zone that is decisive in the assertion that the forest response is strongly correlated to the type of earthworm species in place (Bohlen et al. 2004). Our results and conclusions are based on observational data. There is a need for experimental studies to try to approach the impact of exotic earthworms on forest understory species.

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CHAPITRE 3

Effet des vers de terre exotiques sur les plantes de sous-bois de forêt.

Titre original: Effect of exotic earthworms on forest understory plants (*sera soumis* à Botany)

Le présent chapitre présente une étude expérimentale qui a été réalisée en mettant en place un dispositif expérimental fait de 96 mésocosmes dans lesquels nous avons transplanté 12 espèces herbacées représentatives d'une communauté de sous-bois afin de mesurer l'effet d'un ver de terre introduit sur ces dernières.

Les résultats de cette étude montrent qu'après deux saisons de croissance, seule l'espèce *Phegopteris connectilis* a répondu de façon significative à l'effet des vers de terre et sa biomasse a augmenté en présence de ceux-ci.

De cette étude, on a conclu que si l'effet des vers de terre *L. terrestris* sur les sols dans nos mésocosmes est semblable aux effets observables sur les sols agricoles, il est possible que l'effet positif de cette espèce soit dû à l'augmentation de fertilité causée par l'augmentation de décomposition de la matière organique du sol. Si c'est bien le cas, nous ne pouvons pas expliquer pourquoi les autres espèces n'ont pas répondu de la même façon.

Effect of exotic earthworms on forest understory plants

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Abstract

It has been hypothesized that forests in Canada and the northern United States are experiencing changes in communities of herbaceous forest understory species following an invasion of exotic earthworms. These earthworms were introduced during colonial times into these forests that have existed in the absence of earthworms since the end of the Wisconsin glaciation over 10,000 years ago. In this study we experimentally test if the addition of *Lumbricus terrestris* changes the probability of survival and growth of understory plants and, if so, the mechanisms by which these invasive worms affect the herbaceous plants in these forests. To understand these mechanisms, we chose plant species that have functional traits that can enhance or reduce the hypothetical negative effects created by two environmental changes caused by earthworms. Thus we set up an experimental system made of 96 mesocosms in which we transplanted 12 herbaceous species representative of a community of forest understory selected according to their abundance to ensure that we had enough individuals. The species were chosen according to two traits: (i) the rooting position of the plants (2 levels, either in organic matter or in mineral soil) and (ii) the rate of root mycorrhization (2 levels, strongly mycorrhized or weakly mycorrhized). After two growing seasons, only *Phegopteris connectilis* responded significantly to the effect of earthworms and its biomass increased in their presence. If the effect of earthworms on soils in our mesocosms is similar to the effects on agricultural soils, it is possible that the positive effect of this species is due to increased fertility caused by the increased decomposition of soil organic matter.

Keywords: Earthworms, forest understory plant communities

835

1. Introduction

Human impacts on global ecosystems are now so pervasive that many speak of the Anthropocene era (Sprankling, 2017). One of these impacts is the introduction of exotic species. When the abundance of introduced species becomes significant, and when the impact of these introduced species on these ecosystems becomes major, we speak of invasive alien species. The majority of earthworm species in northern North America are among these invasive alien species. The Wisconsin glaciation decimated almost all of the native earthworms in Canada and the northern United States. The forests of this part of the American continent have evolved in the absence of these worms (Portes 1977, James 1998). Soils containing earthworms in this region, even today, are mainly associated with human activities (agriculture, horticulture, etc.) and their relative abundance and specific composition are strongly linked to soil type, climate, vegetation, topography, land use history and especially its history of exotic species evasions (Hendrix and Bohlen, 2002). Although earthworms are increasingly found in forest environments, there are still several forest areas without earthworms (Hedrix and Bohelen, 2002). This is particularly the case for areas that were not subject to human influence after the glaciation. This is because, in the absence of human introductions, their propagation is only about 5-10 meters per year depending on the region (Addison 2009, Cameron et Bayne 2009).

Earthworms are often referred to as "ecosystem engineers" because they significantly alter the structure of the soil. In particular, they directly modify decomposition rates, the physical structure of the soil and the microbial communities of the soil. Therefore, it is reasonable to believe that the introduction of these species into ecosystems that have developed without these species for more than 10 000 years will have significant impacts. One of the first consequences of introducing earthworms into a forest ecosystem is the reduction of the dead leaf layer (litter). Exotic earthworm invasions have been associated with decreased forest soil thickness and the

development of horizon thickness A (Langmaid 1964; Alban and Berry 1994; Shakir and Dindal 1997; Bohlen et al 2004b; Hale et al, 2005).

Because understory plants vary in the placement of their root systems, it is reasonable to believe that the abundance of understory plant species with these differences in the placement of their root systems will be affected differently by earthworm invasion. For instance, some species that place their roots only in the top layer might be negatively affected while others with roots in mineral soil could not be affected. We also know that earthworms modify the structure of soil horizons, nutrient availability, and soil fauna. Earthworms are part of the detritivore community, consuming leaf litter and increasing decomposition rates. The magnitude of the impacts of earthworm invasions on forest soils depends on the assemblage of earthworm species that invade the soil and their historical use (Frelich et al, 2006).

Finally, the loss of the top layer of forest soils causes changes in the mycorrhizal community. Changes in soil structure and earthworm chemistry and grazing lead to changes in the abundance and structure of soil fungal communities (Johnson et al 1992; McLean and Parkinson, 1998b, 2000). Loss of mycorrhizae can have negative effects on plant root function (Lawrence et al., 2003), plant growth (Gundale, 2002) and plant community assemblages. In addition, an increase in earthworm diversity can lead to a decrease in plant species diversity due to the fact that different earthworm species inhabit several niches in the soil. Hale and colleagues (2006) found lower plant diversity in areas where both epigeic (living in the upper organic layer of the soil) and endogeic (in organic and mineral layers of the soil) worm species are found than in areas where only endogenic worms are found (Hopfensperger et al., 2011). Declining rates of fungi abundance or colonization or changes in the composition of the mycorrhizal fungal community could lead to changes in plant communities in both deciduous and boreal forests, including understory communities (Frelich et al, 2006), which are the focus of this study. Although there are few mycorrhizal plant species, such as *Arisaema triphyllum* and *Circaea lutetiana*, which have a positive relationship with earthworm biomass, a much larger number of plant species are negatively correlated with earthworm biomass (Hale 2004).

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893 We know that the physical, chemical and microbiolnical structure of forest soils are changed as
894 a result of the invasion of earthworms. Several authors have stated that earthworms strongly
895 influence the composition of the herbaceous plant community (Drouin et al, 2014, Frelich et al,
896 2006). Typically, these studies demonstrate an association between the presence or abundance
897 of earthworms and a change in the structure of the plant community. However, an association
898 between earthworm distribution and understory vegetation structure is not necessarily a causal
899 relationship since the correlation between the two can be a common response to human
900 disturbances, even for disturbances that date back several decades. For example, Beauséjour *et*
901 *al.* (2015) concluded that some species were distributed taking into account not only the spatial
902 distribution of human disturbances, but also the time frame within which the disturbance spread.
903 Therefore, there is both correlational evidence and lnical reasons to hypothesize that the
904 invasion of earthworms from forest soils causes a change in the structure and composition of
905 understory plant communities. However, there are two significant weaknesses in all these
906 studies. First, these studies are based on correlations between the presence/absence (or
907 abundance) of earthworms and changes in the composition of plant species. However, our
908 forests are being challenged simultaneously by several changes: forest management, changes in
909 herbivore populations (especially white-tailed deer), nitrogen deposition and climate change. It
910 is very difficult to separate the effects of forest soil invasions by earthworms from these other
911 changes using observational studies. Second, even if we could attribute a causal effect to
912 earthworms, it is likely that this effect will be different on different plant species. Some plant
913 species may be favoured, and others negatively affected. How can these effects be predicted?

914 To test a causal relationship between the introduction of earthworms and changes in the
915 composition of the understory plant community, it is necessary to conduct a field experiment by
916 introducing earthworms into soil that has never had earthworms, with appropriate statistical
917 control, and then monitor the changes in vegetation. Such an experiment has not been done and
918 probably never because of environmental and ethical constraints. Instead, we propose a
919 compromise between such an ideal experiment and purely observational studies: an experiment
920 in which the presence/absence of earthworms is manipulated under controlled conditions that

do not endanger forest soils. In addition, we chose plant species that have functional traits that can enhance or reduce the hypothetical negative effects created by two environmental changes caused by earthworms.

First, the arrival of earthworms in forest soil causes the reduction of the dead leaf layer (litter) on the soil surface, the layer of decomposed organic matter in forest soils. Since many understory species keep their root systems in this layer, we hypothesize that these species will be negatively affected by earthworms. Species which roots are in mineral soil should not be affected as much and may even be advantaged. We also know that earthworms destroy fungal hyphae, including mycorrhizal hyphae (Pelosi et al, 2014) presumably by eating them. If so, understory species that are more heavily mycorrhizal may be more negatively affected by earthworms. In this study we grow 12 understory species having differences in root system placement and mycorrhizal status in experimental mesocosms both in the presence and absence of earthworms.

We have two hypotheses (predictions):

1) Plant species with roots located only in the litter layer will be negatively affected by earthworms. With the loss of litter caused by earthworms, we expect that this category of plants will see its abundance reduced.

2) Plant species that have high rate of mycorrhizas will be negatively affected by the effect of earthworms because of the destruction of their mycorrhizas caused by earthworms.

2. Methods

Experimental design

In order to approach the effects of exotic earthworms on understory plant communities in southern Quebec, we built an experimental system consisting of 96 mesocosms, each 20 cm wide and 50 cm high. We planted the following 12 species, whose names, abbreviations and rates of mycorrhizas determined by using the Brundrett method (1996) are also given in Table 1 (More details in the appendix). In each mesocosm, we put at the bottom 5 cm of garden gravel, then 25 cm of sand and finally a 20 x 20 x 10 (depth) cm cube of soil taken from the forest with the plant a week before the beginning of the experience. For each of the 12 selected plant species, we used individuals of similar size, and for each soil cube we kept only the individual selected for the experiment. The last 10 cm of the mesocosm was left empty to prevent earthworms from escaping. A fine mesh was placed at the lower level of each mesocosm to facilitate the exit of water while blocking the passage of earthworms.

The soil cubes, each with a single plant belonging to one of 12 different species (described below) come from the Johnville Forest Park, (45.347°N, 71.754°W), in the Estrie region of southeastern Quebec, Canada. This park has an area of 224 ha, 85 of which are peat bogs (Grégoire et al, 2010), the rest being forests. This forest is divided into 42 plant stands (M. Dufresne, personal communication, Nature Cantons de l'Est), and the most dominant plants are *Acer rubrum* Linnaeus, *Betula papyrifera* Marshall, *Abies balsamea* Linnaeus, *Betula alleghaniensis* Britton. This is a mixed forest located about 300 km south of the boreal forest. The park prohibits activities that could have a negative impact on wetlands and forests, such as drainage, road construction, motorized traffic, etc., except for all recreational and educational activities intended for the general public (L. DeSerres, personal communication). This site is located in an area that receives up to 1209 mm of precipitation per year and average temperatures range from -10.2°C in January to 19.0° in July (Environment Canada, 2018). After transplanting the plants and their soil cubes, they were photographed (to obtain the surface area of the aerial

parts of each of them), measured (for each plant, only the aerial part from the collar to the terminal burgeon was measured) and the number of leaves per plant was counted. This was used to estimate their initial above-ground biomass. The sampling and transplantation were carried out in May 2017. After two growing seasons, the plants were harvested, dried for a week in an oven at 80 degrees Celsius. The aerial and root parts were weighed with a scale to determine the aerial and root biomass.

Experimental treatments

The experiment included two factors: (i) the presence/absence of earthworms (2 levels) and (ii) the taxonomic identity of the plant (12 species). The earthworm *Lumbricus terrestris* Linnaeus 1758, from the anecic group, was used in this experiment. This choice is explained by its activity of mixing the soil by digging important tunnels and its diet: it feeds on litter (organic matter in general) and destroys mycorrhizae (Pelosi, 2008; Lawrence et al., 2003). It is also one of the most widespread invasive earthworm species in Canada (along with *Eisenia fetida*), found in 11 of Canada's 13 provinces and territories (Addison, 2009).

The 12 herbaceous understory species were chosen from the herbaceous species available on the site, based on their abundance to ensure that there were enough individuals, using two traits: (i) the rooting position of the plants (2 levels, either in organic matter or in mineral soil) and (ii) the rate of root mycorrhization (2 levels, with high rate of mycorrhizas or with low rate of mycorrhizas). Thus, the experiment includes 12 species divided into two categories according to the location of their root system in the different soil horizons: 6 have roots only in the organic layer of the soil and 6 others in the mineral soil and for each category, 3 are highly mycorrhized and 3 others weakly. After selecting our twelve plant species, we classified them according to their mycorrhization rate in decreasing order of magnitude and so the first six are said to have a high mycorrhization rate and the other six have a low mycorrhization rate, although there is no clear limit between these two categories. Each of the 96 individual plants was in its own

mesocosm. There were 8 individuals per species, 4 of which were in each of the 2 levels of "earthworm" treatment.

The classification of root system placement, and root mycorrhization rate was done from an unpublished database of the Shipley Laboratory including 45 species of understory plants. At least 10 individuals of each of these 45 species were observed in nature and the placement of the root system was noted in relation to the organic and mineral layers. The determination of root placement consisted, in the natural environment for each of 45 species to locate in which soil horizon the root system was limited. For our study, we worked with two layers (organic matter and mineral soil). The percent of root fragments that were mycorrhized was determined using the Brundrett method (1996) (See more details in appendix). Table 1 summarizes the experimental design and gives the taxonomic names of the 12 herbaceous species and their mycorrhization rate.

1013 **Table 1. Species according to root placement and mycorrhization rate.**

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Species (Abbreviation)	Placement of root system	Level of mycorrhization (Rate)
Thelypteris noveboracensis (THNO)	Mineral soil	High (88.7%)
Dryopteris intermedia (TRIN)		High (94.7%)
Tiarella cordifolia (TICO)		High (89.8%)
Pyrola elliptica (PYEL)		Low (44.22%)
Cornus canadensis (COCA)		Low (70.9%)
Carex scabrata (CASC)		Low (44.5%)
Trillium undulatum (TRUN)	Organic matter	High (81.1%)
Phegopteris connectilis (PHCO)		High (91%)
Streptopus lanceolatus (STLA)		High (82.2%)
Lycopodium annotinum (LYAN)		Low (34.9%)
Smilacina racemosa (SMRA)		Low (59.8%)
Arisaema triphyllum (ARTR)		Low (59%)

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Application of treatments

A shade cloth that lowers daylight (a decrease of about 84%) was placed above the mesocosms. The light intensity measured at the soil surface ranged from 66.76 to 43.35 micromoles/m^2/s depending on the conditions. We added 10 individuals of *Lumbricus terrestris* in each of the 48 mesocosms with the "earthworm" treatment in early June 2017 using random numbers. This is a density of 250 individuals per m^2, which is a realistic density according to an unpublished study conducted by Ndabarushimana and Shipley in 2018.

To ensure the absence of worms in "earthworm-free" mesocosms, a mustard solution (10 g/L) was applied at the beginning of the experiment (June 2017) and in the spring of the following year (June 2018). Each mesocosm received about 2 liters of solution. All earthworms that rose to the surface within the next 30 minutes were removed. All mesocosms were watered after this treatment to dilute the mustard solution poured into the mesocosms to mitigate possible effects of this solution, but there was probably mustard that remained in the soils of this treatment. We decided not to use this mustard solution on mesocosms with the addition of earthworms since it is impossible to completely remove the mustard, since this mustard remains in the soil for an unknown period of time, and since this mustard, if applied in mesocosms with earthworms, would have caused the added earthworms to flee. It is important to note that the two levels of treatment are actually "with earthworms and without mustard" versus "without earthworms and with mustard". Since the application of this solution forces earthworms to rise to the surface (Pelosi et al, 2014), we were able to ensure that earthworms were absent in these mesocosms without disturbing the soil and plants. In fact, we found earthworms in few mesocosms (in 6 mesocosms and their numbers varied from 1 to 3 individuals).

Finally, we regularly watered the soil to keep it moist, to minimize transplant shock, and to mitigate any effects that the mustard solution might cause.

Initial and final above-ground biomass measurement

In order to estimate the initial biomass of each transplanted plant, we developed prediction equations based on observational measurements of the plants. To do this, we selected about 30 additional individuals of each species from the same site. These individuals were chosen to maximize the variation in above-ground biomass within each species. We then measured the following features:

- The total leaf area: this measurement was obtained by analyzing the photos of the aerial parts of these individuals using the CellProfiler software (Version 2.2). The height of the photo taking remained constant for all individuals photographed.
- Vegetative height: This is the height of each individual, measured from the collar to the top.
- Dry biomass: Each individual was then cut at the soil surface and dried for a week in an oven at 80°C and its dry weight measured.

Thus, for each species the model: lm(ln(ln(Dry Biomass)~ln(Height)+ln(Total leaves area)+ln(Number of leaves)...) led us to its prediction equation

1062 **Table 2. Prediction equation of the initial biomass of twelve species used in the experiment.**

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Species (Abbreviation)	Equation
Thelypteris noveboracensis (THNO)	ln of dry biomass = - 9.093019 + 2.082195 x ln (Vegetative height) + 0.05117 x ln (The total leaves area) x (Number of leaves)
Dryopteris intermedia (TRIN)	ln of dry biomass = - 5.61168 + 0.93825 x ln (The total leaves area)
Tiarella cordifolia (TICO)	ln of dry biomass = - 5.68313 + 0.37825 x ln (Vegetative height) + 0.66727 x ln (The total leaves area) + 0.23026 x (Number of leaves)
Pyrola elliptica (PYEL)	ln of dry biomass = - 8.022 + 1.66446 x ln (Vegetative height) + 0.69734 x ln (The total leaves area) + 0.53998 x (Number of leaves) - 0.20327 x ln (Vegetative height) x (Number of leaves)
Cornus canadensis (COCA)	ln of dry biomass = - 6.18072 + 1.01954 x ln (The total leaves area) + 0.0776 x (Number of leaves)
Carex scabrata (CASC)	ln of dry biomass = - 4.70292 + 0.86367 x ln (The total leaves area)
Trillium undulatum (TRUN)	ln of dry biomass = -6.93174 + 1.09955 x ln (The total leaves area)
Phegopteris connectilis (PHCO)	ln of dry biomass = 0.4575 - 2.35051 x ln (Vegetative height)-0.5111 x ln (The total leaves area) - 3.24168 x (Number of leaves) +0.49876 x ln (Vegetative height) x ln (The total leaves area) + 1.26488 x ln (Vegetative height) x (Number of leaves) + 0.58708 x ln (The total leaves area) x (Number of leaves) -0.22242 x ln (Vegetative height) x ln (The total leaves area) x (Number of leaves)
Streptopus lanceolatus (STLA)	ln of dry biomass = - 6.59805 + 1.12396 x ln (The total leaves area)
Lycopodium annotinum (LYAN)	ln of dry biomass = - 3.18807 + 0.87583 x ln (Vegetative height) + 0.09965 x (Number of leaves)
Smilacina racemosa (SMRA)	ln of dry biomass =-7.2892 + 1.1958 ln (Vegetative height) + 0.6551 x ln (The total leaves area)
Arisaema triphyllum (ARTR)	ln of dry biomass = -7.843 + 1.1925 x ln (Vegetative height) + 0.6312 x ln (The total leaves area)

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3. Statistical analyses

All statistical analyses were performed with R version 3.3.3 software (R Development Core Team, 2017). Plant survival in mesocosms was modeled using generalized linear models using the glm function and using the binomial distribution to our model (Crawley, 2012). The model used is: glm (survival~species+treatment+species:treatment, family=binomial).

In our analyses of above-ground biomass, we used the natural ln transformation in our statistical model since it is under these conditions that we obtained a residual curve with a normal distribution. The response variable is the increase in above-ground biomass: lm(ln(final biomass)-ln(initial biomass)). The statistical model is lm(increase~species+treatment+species:treatment).

We tested the statistical significance of the relationships in these generalized linear models between plant survival and variables such as species and treatment with type II ANOVAs using the chi-square distribution and comparing the nested models (with/without the explanatory variable). For biomass models, we also used type II ANOVAs using the Fisher F distribution and comparing nested models (with/without the explanatory variable). We used a Tukey post-hoc test to identify significant differences.

4. Results

Survival:

There were relatively few deaths during the experiment. Of the 96 plants, 9 died and 87 survived to the end of the experiment. *Pyrola elliptica* had the highest mortality rate, at 3 out of 8 individuals, followed by *Lycopodium annotinum* and *Cornus canadensis*, which lost 2 individuals

each, and finally *Tiarella cordifolia* and *Carex scabrata*, which lost 1 individual each. The rest of the species did not have any mortality. These cases of mortality all occurred after at least 8 weeks after the initial transplant. Of the 9 dead, 7 out of 9 were in mesocosms without earthworms. The two-way ANOVA shows that there are differences between species (Df = 1, Deviance = 19.8327, P=0.0477). In addition, plants in the "no earthworm" treatment showed a higher mortality rate (Df = 1, Deviance = 3.9617, P=0.0466, Figure 4). There are no interactions between the effect of the treatment and the species (Df = 11, deviance = 5.1839, P=0.9219).

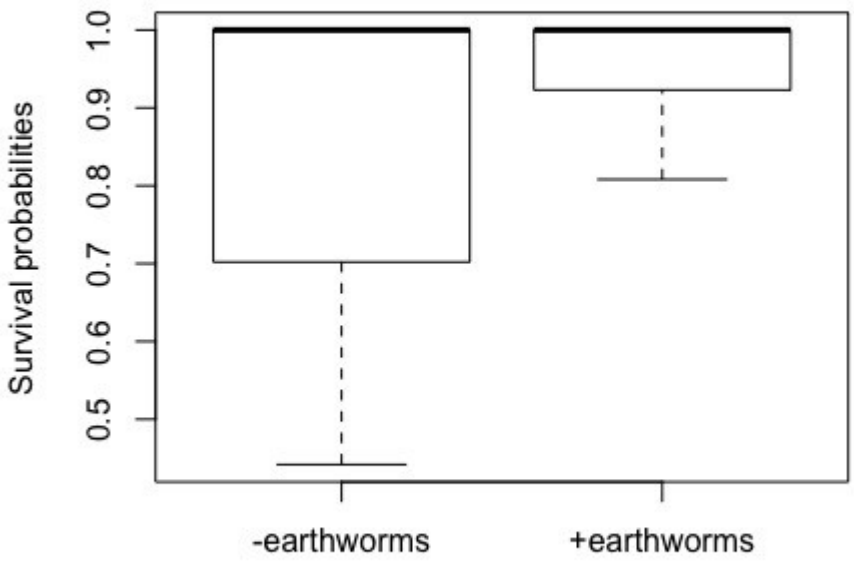

Figure 4. Probabilities of survival of species with or without earthworms.

Growth:

The experiment, which began in May 2017, was closed after two growing seasons in August 2018.

After these two growing seasons and taking into account the initial differences in above-ground biomass, it can be seen that all plants grew during the experiment.

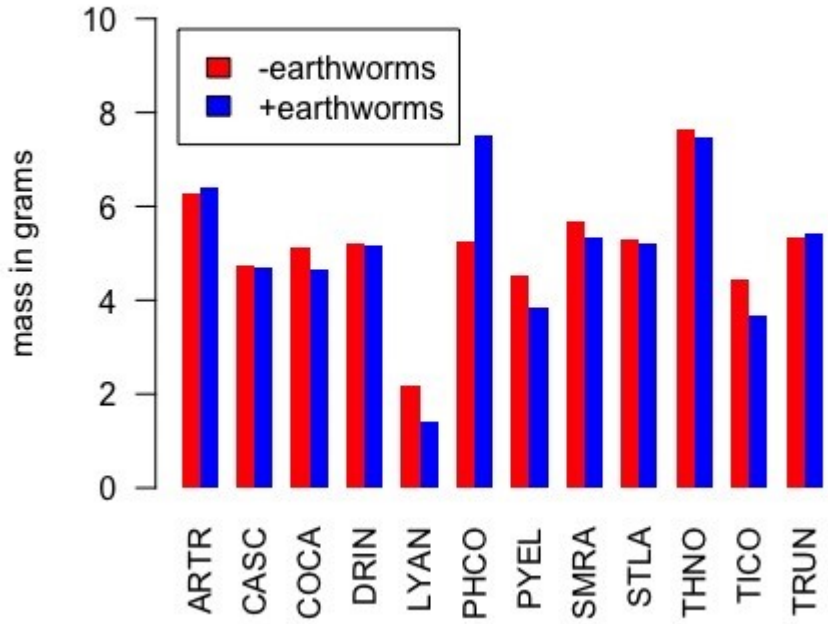

Figure 5. Species growth by treatment type.

ARTR = *Arisaema triphyllum,* CASC= *Carex scabrata*, COCA = *Cornus canadensis*, DRIN = *Dryopteris intermedia*, LYAN = *Lycopodium annotinum*, PHCO = *Phegopteris connectilis*, PYEL = *Pyrola elliptica*, SMRA = *Smilacina racemosa*, STLA = *Streptopus lanceolatus*,

1116 THNO = *Thelypteris noveboracensis*, TICO = *Tiarella cordifolia*, TRUN = *Trillium*
1117 *undulatum*.

1118

1119 The analysis of the variance of the biomass increase detected a significant interaction (species
1120 x treatment, $F_{11,63} = 3.6278$, $P = 0.0005$). So, the treatment had an effect, but the strength of that
1121 effect varies from species to species. Figure 5 summarizes these trends. Of the 12 species
1122 selected for this experiment, only *Phegopteris connectilis* responded significantly to the
1123 treatment of adding earthworms ($P < 0.0001$), by growing more.

1124

1125 **Table 3. Statistical results of multiple ANOVAs to determine the effect of earthworms**
1126 **depending on the plant species.**

1127

Species (Abbreviation)	contrast	estimate	df	t. ratio	p. value
Thelypteris noveboracensis (THNO)	(+earthworm) - (-earthworm)	-0.03706228	63	-0.091	0.9280
Dryopteris intermedia (DRIN)	(+earthworm) - (-earthworm)	0.09617360	63	0.235	0.8146
Tiarella cordifolia (TICO)	(+earthworm) - (-earthworm)	-0.61088330	63	-1.385	0.1710
Pyrola elliptica (PYEL)	(+earthworm) - (-earthworm)	-0.56967438	63	-0.882	0.3811
Cornus canadensis (COCA)	(+earthworm) - (-earthworm)	-0.35825531	63	-0.760	0.4503
Carex scabrata (CASC)	(+earthworm) - (-earthworm)	0.10988062	63	0.249	0.8041
Trillium undulatum (TRUN)	(+earthworm) - (-earthworm)	0.19987772	63	0.489	0.6263
Phegopteris connectilis (PHCO)	(+earthworm) - (-earthworm)	2.40030329	63	5.876	<.0001*
Streptopus lanceolatus (STLA)	(+earthworm) - (-earthworm)	0.03732024	63	0.085	0.9329
Lycopodium annotinum (LYAN)	(+earthworm) - (-earthworm)	-0.64252549	63	-1.456	0.1503
Smilacina racemosa (SMRA)	(+earthworm) - (-earthworm)	-0.20236417	63	-0.495	0.6220
Arisaema triphyllum (ARTR)	(+earthworm) - (-earthworm)	0.12834664	63	0.314	0.7544

5. Discussion

Following the lnic that earthworms reduce the dead matter layer on the soil surface, and destroy mycorrhizal hyphae, we proposed two hypotheses: (1) species rooted only in the litter layer will be adversely affected and (2) species that are highly mycorrhized may be negatively affected. Based on our results, both of these hypotheses must be rejected.

Although the effects of earthworms did not follow our assumptions, our results suggest that earthworms can affect the probabilities of survival and growth of understory species since we observed significant effects of manipulating the presence/absence of earthworms on survival and growth. Therefore, it is possible that the invasion of forest soils by earthworms can change the structure of forest understory plant communities. However, contrary to our expectations, plants had a better chance of survival when they were in mesocosms containing earthworms than in the mesocosms in which we removed earthworms after applying the mustard solution.

Most research on the effects of earthworms on ecosystem functioning has been conducted in agricultural systems, where earthworms are credited with improving soil structure and increasing nutrient cycle rates (Lee 1995; Lavelle et al. 1998; Parmelee et al. 1998; Hale et al. 2005). Indeed, earthworms strongly affect the availability of soil nutrients, either directly through soil transformation or indirectly through changes in the microbial and invertebrate communities of the soil, but exotic earthworm invasions have variable effects on nutrient availability. Laboratory and microcosm field studies have shown that the presence of earthworms (Frelich et al, 2006; Scheu 1987; Scheu and Parkinson 1994a, b) increases nutrient availability and plant growth.

The species *Phegopteris connectilis* is the only one that responded significantly to the effect of earthworms and its biomass increased in the presence of earthworms. If the effect of earthworms on soils in our mesocosms is similar to the effects on agricultural soils, it is possible that the positive effect of this species is due to the increased fertility caused by the increased decomposition of soil organic matter. If so, we cannot explain why other species did not respond

in the same way. However, herbaceous understory species are all perennial species that grow rather slowly, although all species showed an increase in biomass during the experiment. If the effect of earthworms is small, it is possible that two growing seasons may not be sufficient to demonstrate the effects of earthworms on the majority of our species.

As explained in the Methods section, the only way to ensure the total lack of earthworms in the "no earthworm" treatment without disturbing the natural soils with its plant was to apply the mustard solution. Also, since we cannot add this solution in the "with earthworm" treatment without disturbing the earthworms, this leaves the possibility that the addition of mustard, rather than the direct handling of the earthworms, could have caused the slight increase in mortality and slight decrease in growth for a species in the "without" treatment. Without completely excluding this possibility, we do not believe that this is the case. First, the amount of mustard added per mesocosm was minimal. Secondly, we do not know of any mechanism by which mustard powder will cause mortality or reduce the growth of adult plants. The main secondary compounds in mustard are glucosinolates. Cipollini and Cipollini (2016) reviewed the literature on the allelopathic effects of an invasive mustard species in America (*Alliaria petiolata*) on the germination of other species and the survival of underbrush plants, as well as on bacterial and fungal communities. Their conclusion is that these allelopatic effects in nature are minimal or do not exist.

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CHAPITRE 4

DISCUSSION ET CONCLUSION GÉNÉRALE

L'invasion des vers de terre exotiques dans les forêts tempérées et boréales en Amérique du nord, longtemps ignorée, est devenue une préoccupation grandissante dans le milieu scientifique (Hale et al., 2005; Suarez et al 2006; Addison 2009). Cette problématique d'invasion de vers de terre en sols forestiers du Canada et du nord des États-Unis est actuellement un sujet sérieux qui devrait capter l'attention de plus d'un. Les études déjà faites dans le passé suggèrent que ceci est un problème réel. Cependant, on ne doit pas ignorer que la plupart des études faites jusque-là présentent deux faiblesses importantes.

D'abord, ces études se basent sur des corrélations entre la présence/absence (ou abondance) des vers de terre et des changements de composition des espèces végétales. Par contre, nos forêts sont bousculées simultanément par plusieurs changements : l'aménagement forestier, les changements dans les populations d'herbivores (surtout les cerfs de virginie), les dépositions azotées et les changements climatiques. Il est très difficile de séparer les effets des invasions des sols forestiers par les vers de terre de ces autres changements en utilisant les études observationnelles. Ensuite, même si nous pouvions attribuer un effet causal aux vers de terre, il est probable que cet effet sera différent sur différentes espèces végétales. Certaines espèces végétales peuvent être favorisées, et d'autres affectées négativement. Comment prévoir ces effets?

Pour démontrer une relation causale entre l'introduction des vers de terre et des changements dans la composition de la communauté des plantes de sous-bois, il faudra faire une expérience sur terrain en introduisant des vers de terre dans un sol qui n'a jamais eu des vers de terres, avec un contrôle statistique approprié, et ensuite suivre les changements dans la végétation. Une telle

expérience n'a pas été faite et ne sera probablement jamais faite à cause des contraintes d'éthique environnementale.

Les résultats de l'étude que nous avons réalisé par le biais de l'échantillonnage (Chapitre 1) ont conclu que la distribution des vers de terre et la structure des communautés d'espèces de sous-bois de forêt du parc écoforestier de Johnville suivent l'historique de l'utilisation de ce site. Et puis dans le deuxième chapitre nous avons pu nous rendre compte de l'évidence de la capacité de vers de terre à influencer la croissance et la survie des espèces de sous-bois même si nous n'avons pas été à mesure prédire la catégorie d'espèces herbacées qui pourra être affectée positivement ou négativement.

En outre, l'étude menée par Drouin et *al* (2016) a également permis de confirmer que la distribution et l'abondance des vers de terre exotiques observées en forêts sont le reflet de l'accessibilité humaine à ces milieux et que leur dispersion est facilitée par les humains car des vers de terre ont été retrouvés dans des sites non protégés et traversés par des sentiers et aucun ver n'a été trouvé sur des sites protégés ou sites éloignés des sentiers et des habitations. Cette étude a également suggéré que les conséquences de la propagation croissante des vers de terre dans la forêt tempérée pourraient compromettre l'intégrité de la biodiversité et le recrutement des espèces d'arbustives. L'étude en question a mis en évidence une corrélation négative entre l'abondance des vers de terre et l'abondances de certaines espèces de plantes.

Cependant, on sait aussi que l'historique d'utilisation des sites peut expliquer la distribution et la vitesse de dispersion des vers de terre. Les résultats de Beauséjour *et al* (2015) ont conclu que certaines espèces étaient distribuées en tenant compte non seulement de la distribution spatiale des perturbations humaines, mais aussi le délai dans lequel la perturbation s'est répandue. Certaines des perturbations les plus anciennes sur leur site d'étude (Mont Saint Hilaire) sont à peine évidentes dans le domaine à l'heure actuelle, mais leur influence persiste jusqu'à nos jours.

Donc, l'historique des sites forestiers (anciennes terres agricoles ou non) doit être pris en compte pour comprendre la distribution actuelle.

De ce qui précède, il est évidant que cette problématique de vers invasifs nécessite encore plus d'investigations pour pouvoir comprendre et prévoir avec certitude les effets de vers afin de formuler des recommandations aux gestionnaires des forêts visées par cette invasion.

ANNEXES

A. Communauté végétale échantillonée à Johnville

Les espèces qui forment la canopée sont les suivantes : *Abies balsamea, Acer pensylvanicum, Acer rubrum, Acer saccharum, Acer spicatum, Alnus sp., Betula alleghaniensis, Betula papyrifera,Fagus grandidentata,Fraxinus americana,Pinus banksiana, Picea sp., Pinus strobus, Populus balsamifera, Populus deltoides, Populus grandidentata, Populus tremuloides, Prunus serotina, Prunus sp., Rhamnus sp.,Thuja occidentalis, Tsuga canadensis , Ulmus americana, Ulmus rubrum.*

Les espèces de sous-bois qui ont été échantillonnées dans nos placettes puis identifiées sont les suivantes (Leurs codes sont données entre crochets) :

Agrimonia striata Michx [AGST], *Arisaema triphyllum (L.) Scott.* [ARTR], *Aster sp.* [ASSP], *Athyrium filix-femina (L.) Roth* [ATFI], *Botrychium dissectum Sprengel* [BODI], *Carex scabrata* [CASC], *Caulophyllum thalictoroides L.* [CATH], *Circaea alpina L.* [CIAL], *Clintonia borealis (Ait.) Raf.* [CLBO], *Clematis virginiana L.* [CLVI], *Coptis trifolia (L.) Salisbury* [COTR], *Dalibarda repens L.* [DARE], *Dryopteris phegopteris (L.) C. Chr.* [DRIN], *Dryopteris spinulosa (O. F. Muell.) Watt.* [DRSP], *Epipactis helleborine (L.) Crantz.* [EPHE], *Equisetum sp.* [EQSP], *Eutrochium maculatum* [EUMA], *Fragaria virginiana Duchesne* [FRVI], *Gallium sp.* [GASP], *Hieracium aurantiacum* [HIAU], *Impatiens capensis Meerb* [IMCA], *Linnaea borealis L.* [LIBO], *Lycopodium clavatum L.* [LYCL], *Lycopodium obscurum L.* [LYOB], *Maïanthemum canadense Desf.* [MACA], *Matteucia sp.* [MASP], *Onoclea sensibilis L.* [ONSE], *Oxalis stricta L.* [OXST], *Pteridium aquilinum (L.) Kuhn* [PTAQ], *Pyrola sp.* [PYSP], *Ranunculus abortivus L.* [RAAB], *Rubus sp.* [RUSP], *Sedum purpureum* [SEPU], *Sphaignes, Taxacum sp.* [SPTA], *Thelypteris hexagonoptera* [THHE], *Thelypteris noveboracensis (L.) Nieuwl.* [THNO], *Thelypteris palustris Schott var. pubescens*

(Lawson) Fernald [THPA]*, Tiarella cordifolia L.* [TICO]*, Trientalis borealis Raf.* [TRBO]*, Trillium undulatum, Violette sp.* [TRUN].

B. Espèces utilisées dans l'expérience ainsi que leur taux de mycorhization

Thelypteris noveboracensis (L.) Nieuwl. (en), 1910 (THNO, 88.7%), *Dryopteris intermedia* A. Gray, 1848 (DRIN, 94.7%), *Tiarella cordifolia* L. (TICO, 89.8%), *Pyrola elliptica* Nuttall, 2009 (PYEL, 44.22%), Cornus canadensis L., 1758 (COCA, 70.9%), *Carex scabrata* Schwein (CCAC, 44.5%), *Trillium undulatum* Willd.., 1801 (TRUN, 81.1%), *Phegopteris connectilis* Watt, 1866 (PHCO, 91%), *Streptopus lanceolatus* Reveal, 1993 (STLA, 82.2%), *Lycopodium annotinum* L., 1753 (LYAN, 34.9%), *Smilacina racemosa* L. (SMRA, 59.8%), *Arisaema triphyllum* Schott, 1832 (ARTR, 59%)

C. Protocole de détermination du taux de mycorhization

Pour les AMF (Arbuscular mycorrhizal fungi):

1- Rincer les racines à l'eau jusqu'à ce qu'elles soient bien propres
2- Les faire tremper dans du KOH 10% (p/v). Le temps de trempage varie d'une espèce à l'autre (certaines racines, comme celles des plantes ligneuses, vont demander plus de décoloration). Aussi, certains autoclavent leurs racines dans le KOH. Ce n'est pas toujours nécessaire pour beaucoup d'herbacées, on peut les laisser même tremper à T° de la pièce pendant ~ 1 semaine. Une autre méthode à regarder est celle de Dalpé (2013), Mycorrhiza, avec chauffage au micro-ondes: rapide, efficace, et pour les racines "coriaces", Dans le cas si on pourra rapidement enlever le KOH sale, et décolorer à nouveau si nécessaire. L'idée est que tant que le KOH sort colorer de la décoloration, on doit continuer.

3- Neutraliser le KOH en rinçant avec du vinaigre (e.g., tremper dans le vinaigre pendant 5 min)

4- Colorer avec une solution encre:vinaigre 5% (v/v) pendant 15 min. Certains colorent plus longtemps, encore une fois, ça dépend des espèces.

5- Rincer les racines à l'eau du robinet pour enlever le surplus d'encre.

6- Étaler les fragments racinaires de façon parallèle sur une lame de microscope, et mettre un "mounting medium" temporaire, ou même, pour économiser l'argent, du sucrose 60% (p/v), et couvrir d'une lamelle.

7- Observer à grossissement 100X minimum, parfois plus si nécessaire pour bien distinguer les structures. Pour quantifier la colonisation, suivre McGonigle et al. (1990), New Phytol. (Guideline intersect method). En gros, compter le nombre d'intersections de racines dans notre champ de vision pour lesquelles il y a des AMF (quantification de la FRÉQUENCE). Certains donnent, pour chaque intersection, un score d'ABONDANCE d'AMF à chaque intersection.

Pour les (ECM) ectomycorrhizal plants:

1- Rincer les racines

2- Observer au binoculaire. Compter la proportion de "tips" racinaires pour lesquels on observe des structures caractéristiques des ECM, soit le manteau en particulier.

Brundrett, 1996. Working with mycorrhizas in forestry & Agriculture, cet ouvrage donne plus de details.

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